PATENT

UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 00-1278)

In the Application of: **Lawton, et al.**

Serial No.: **09/765,739**

Filed: **January 18, 2001**

Art Unit: 1645

Examiner: V. Ford

For: **Compositions and Methods for Detection of *Ehrlichia canis* and *Ehrlichia chaffeensis* Antibodies**

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

1. I, Ramaswamy Chandrashekar, am a research scientist for IDEXX Laboratories, Inc., the entire Assignee of U.S. Pat. Appl. Ser. Nos. 09/765,739, 10/054,354, and 10/054,647. I have earned a Ph.D. in Biochemistry and M.S. in Zoology. I have performed research and development in the field of sero-diagnostics for veterinary bacterial pathogens, including, for example, *Ehrlichia* ssp. and *Streptococcus equi* for over five years. In addition, I have performed research and development in the field of sero-diagnostics for nematode infections in both humans and animals for over 20 years. I am an author of over 70 scientific publications in the field of diagnosis and prevention of nematode infections. A copy of my Curriculum Vitae is attached.

2. Waner *et al., (J. Vet. Diagn. Invest.*, Vol. 12, pp. 240-244, 2000), Cadman *et al., (The Veterinary Record,* 135, 362), Rikihisa (WO 99/13720), and other references

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that teach or suggest the use of entire *E. canis* or *E. chaffeensis* infected cells, whole (*i.e.*, non-fragmented) *E. canis* or *E. chaffeensis* proteins, including mixtures of whole proteins, natural whole proteins, or whole recombinant proteins, to, *e.g.*, detect *Ehrlichia,* do not teach or suggest polypeptides of SEQ ID NOs:1-7 to, *e.g.*, detect *Ehrlichia.* As explained in the specifications of the above-mentioned patent applications, entire *E. canis or E. chaffeensis* infected cells, or whole (i.e., non-fragmented) *E. canis* or *E. chaffeensis* proteins, including mixtures of whole proteins, natural whole proteins or whole recombinant proteins are impure reagents, which are of limited usefulness in sero-diagnosis due to sensitivity and specificity issues. For instance, Example 1 of the 09/765,739 application demonstrates that assays that use SEQ ID NOs:1 and 2 were more sensitive and specific than assays that use partially purified *E. canis* antigens. *See e.g.*, paragraph spanning page 20 and 21 of the 09/765,739 application. The partially purified *E. canis* antigens were obtained from *E. canis* organisms grown in tissue culture and partially purified by differential centrifugation and column chromatography. These partially purified *E. canis* antigens were therefore, mixtures of whole proteins.

3. Assays for detecting anti-*Ehrlichia* antibodies or fragments as described by Waner, Cadman, Rikihisa, and others are severely limited in usefulness because of sensitivity and specificity issues directly related to the impure nature of the *Ehrlichia* antigen used in these tests. *See e.g.*, page 2, line 21 through page 3, line 2 of the 09/765,739 specification (emphasis added). The instant inventions provide highly purified reagents for the detection *Ehrlichia*, that is, polypeptides of about 18-20 amino acids. The use of SEQ ID NOs:1-7 instead of the impure reagents described above, to for

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example, detect *Ehrlichia* provide distinct advantages such as greater sensitivity and specificity in sero-diagnostic assays.

4. Waner teaches that the disclosed ELISA assay for detection of *E. canis* closely correlates to the "gold standard" IFA test. *See e.g.*, p. 243, left col. first full paragraph; page 243, right col., first and second paragraph; page 240, right col., first full paragraph.

5. Cadman teaches that the disclosed dot-blot enzyme linked immunoassay (DBELIA) had a sensitivity of 92% and a specificity of 96% when compared to the IFA. See page 135, paragraph spanning columns. Cadman states that the "study showed the DBELIA to be as sensitive and specific as IFA for the detection of antibodies to *E. canis*." See last paragraph.

6. The polypeptides claimed in the instant application have a sensitivity of 98.5% and a specificity of 100% when compared to western blot analysis. Western blot analysis is more sensitive and more specific than IFA analysis. The IFA had, at one time, been considered the "gold standard" for sero-diagnosis of *Ehrlichia*. However, western analysis is more sensitive and more specific than IFA analysis, which uses whole cells as the antigen resulting in cross-reactivity, specificity, and sensitivity issues. The IFA disclosed in the instant invention had a sensitivity of 88% and a specificity of 0%. The polypeptides of the instant invention perform better than the "gold standard" IFA in this study. The Waner and Cadman assays, however, perform only as well as the IFA. Therefore, one of skill in the art could reasonably conclude that the polypeptides of the instant invention perform better, *i.e.*, provide more sensitive and specific results in sero-diagnostic assays, than the Waner and Cadman assays.

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7. Rikihisa teaches the use of recombinant, whole proteins to detect *Ehrlichia* antibodies. Rikihisa does not disclose the sensitivity or specificity of the whole, recombinant proteins in sero-diagnostic assays. However, Ohashi *et al.* (J. Clin. Microbiol. 36:2671 (1998)) (copy attached) teaches that dot blot assays performed with whole *E. canis* rP30 antigen to detect *E. canis* were as sensitive as an IFA assay, specificity was not examined in this study. See page 2678, right column, first full paragraph. The instant invention provides peptides (SEQ ID NOs:1-7) that can provide results that are more sensitive than IFA assays. Therefore, one of skill in the art could reasonably conclude that the peptides of the instant invention are more sensitive and more specific than the antigens reported in Waner, Cadman, and are more sensitive than the antigens reported in Rikihisa.

8. The pure reagents described in the instant inventions have additional advantages as compared to the impure reagents described in Waner, Cadman, and Rikihisa. For example, in experiments performed at IDEXX Laboratories mixtures of SEQ ID NOs:1 and 2 showed no cross-reactivity to *Borrelia burgdorferi, A. phagocytophilum*, and uninfected canine serum. See Table 1.

Table 1.

Peptide	**# of Samples**	**Canine Serum**	**Reactivity**
Mixtures of SEQ ID NO:1 and SEQ ID NO:2	157	Uninfected	0/157
	81	*E. canis*	81/81
	166	*Borrelia burgdorferi*	0/166
	29	*A. phagocytophilum*	0/29

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9. I hereby certify that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 02/13/2004 By: _____

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